REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-16 and 19-35 are in this case.

Claims 5 and 23-35 were withdrawn by the Examiner from consideration as drawn to a non-elected invention.

Claims 1,3 and 7-11 have been rejected under 35 U.S.C. §112, second paragraph.

Claims 1,3,6-13, 15 and 22 have been rejected under 35 U.S.C. §102(b).

Claims 1,3,6-13, 15 and 22 have been rejected under 35 U.S.C. §102 (a).

Claims 1,3,4,6-15 and 19- 22 have been rejected under 35 U.S.C. §103 (a).

Dependent claims 3, 9, 10 and 11 have been amended. Amendments are purely linguistic and do not introduce new matter.

The claims before the Examiner are directed toward vectors for expressing heterologous peptides at the amino-terminus of Potyvirus Coat Protein, methods for use thereof, plants infected with same and methods of vaccination using same.

§ 112, Second Paragraph Rejections

The Examiner has rejected claims 1,3,7-11 and under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Specifically, the Examiner has asserted that claims 1,3,10 and 11 are indefinite because of disagreement between "a heterologous nucleic acid" and "at least a portion of the heterologous peptide". Claims 3, 10 and 11 have been amended so that the antecedent basis

of heterologous peptide is absolutely clear. These amendments are purely linguistic and no introduction of new matter has occurred.

The Examiner's rejection of claims 1,3,10 and 11 under 35 U.S.C. §112, second paragraph is traversed.

The Examiner has further asserted that claims 1,7 and 8 are indefinite because of the phrase "at least one amino acid residue". The Applicant respectfully invites the Examiner to read the three claims in question more closely. Claim 1, as the independent claim, is necessarily the broadest in scope and does not require the presence of an amino terminal domain of the coat protein. Applicant stresses that "amino-terminus" is not synonymous with "amino-terminal domain".

The term "terminus" was specifically employed to designate the extreme end of the peptide. Such usage is consistent with the dictionary definition of "terminus:" which is "an end; final point; extremity or goal" [Webster's New World Dictionary; Second College Edition (1976) William Collins & World Publishing Inc; D.B. Guralnik (editor)].

Further, the term "terminus", whether amino- or carboxy-, is commonly employed by those of ordinary skill in biochemistry to denote an end of a peptide or protein.

Thus, the choice of the term "amino-terminus" was made to "to particularly point out and distinctly claim the subject matter which Applicant regards as the invention" as required by 35 U.S.C. §112, second paragraph.

Claim 7 depends from claim 1 and limits the scope thereof so that the vector is defined, for the first time, as including an "amino-terminal domain". Again, "amino-terminal domain", as opposed to "amino-terminus", is readily understood by those of ordinary skill in the art of virology of potyviridae. Applicant has provided, solely in order to expedite prosecution, pages 121-127 of the standard reference text "Shukla, D.D., Ward, C.W. & Brunt, A.A.; The Potyviridae (1994) Wallingford,UK, CAB International.516 p." [see Appendix A]. The

Examiner is specifically referred to page 121 and to Table 5.1 in which AA sequences of the amino-terminal domain, including the amino-terminus of several potyviruses including ZYMV are set forth. Applicant respectfully points out that since this standard text was widely available more than seven years prior to the filing date of the instant application, it is reasonable to assume that one of ordinary skill in the art would understand the terminology as used therein.

Applicant notes that, owing to an earlier restriction requirement, the potyvirus of the vector is ZYMV. The portion of ZYMV which comprises the amino-terminal domain is well known to those of ordinary skill in the art for many years as set forth hereinabove.

Thus, claim 8, which further limits claim 7 by stating "...wherein said amino-terminal domain is modified by deletion of at least 1 amino acid residue." includes a vector in which any number of residues are deleted so long as at least one residue of the recognized amino terminal domain remains. Because the definition of amino-terminal domain is concrete in the mind of those ordinarily skilled in the art, the language of claim 8 is not indefinite.

In summary, claim 1 includes both vectors with an amino-terminal domain and also those that lack such a domain. Claim 7 includes only those vectors that include an amino-terminal domain. Claim 8 makes it clear that deletions from the amino-terminal domain do not remove the vector from the scope of the claims. For the record, Applicant states that as long as one amino acid residue of the amino-terminal domain remains, the vector is claimed under claims 1, 7 and 8. If no amino acid residue of the amino-terminal domain remains, the vector is claimed under claim 1. If the entire amino-terminal domain is present, the vector is claimed under claims 1 and 7.

The Examiner's rejection of claims 1, 7 and 8 under 35 U.S.C. §112, second paragraph is traversed.

The Examiner has further asserted that claims 1,3 and 9 are indefinite because of the phrase "influences a biological activity". The Examiner asserts that it is unclear that it is the biological activity of the [at least a portion of the] heterologous peptide that is modified. Such an assertion is untenable in the face of the language of claim 9, which is completely unambiguous in this regard. Claims 1 and 3 do not contain the [allegedly] indefinite phrase.

The Examiner's rejection of claims 1, 3 and 9 under 35 U.S.C. §112, second paragraph is traversed.

All rejections under 35 U.S.C. §112, second paragraph are traversed.

§ 102(b) Rejections – Fernandez-Fernandez

The Examiner has rejected claims 1,3,6-13,15 and 22 under §102(b) as being anticipated by Fernandez-Fernandez et al. (Federation of European Biochemical Societies, 1998; hereinafter Fernandez).

The Applicant stresses that Fernandez teaches insertion of heterologous sequence(s) between coat protein residues Ala_{12} and Leu_{13}. Thus Fernandez does not teach, hint or fairly suggest that insertion of a heterologous sequence at the amino-terminus, as instantly claimed, is necessary, advantageous, desirable or even feasible. Arguments concerning the meaning of "amino-terminus" are set forth in detail hereinabove. By definition, any insertion of "heterologous sequence(s) between coat protein residues Ala_{12} and Leu_{13}" will not be at the "amino-terminus" as instantly claimed.

The Applicant was aware of Fernandez earlier work which is reviewed on page 5 of the specification as filed. Applicant reiterates that *"This [Fernandez's] insertion did not*

involve a deletion of any part of the PPV authentic CP-NT nor was the heterologous peptide fused to the extreme N- terminus."

By contrast, the claims before the Examiner are limited to those vectors which include "*a heterologous nucleic acid sequence inserted at the amino-terminus of the potyvirus coat protein.*" [claim 1]. Applicant stresses that amino terminal domain and amino-terminus are not synonymous as described in detail hereinabove. See also claim 2 and page 8; last paragraph:

"According to further features in preferred embodiments of the invention described below, the amino-terminus is selected from the group consisting of: (i) an established amino-terminus of a wild type potyvirus coat protein; and (ii) an alternate amino-terminus of a potyvirus coat protein, the alternate amino-terminus arising from an action selected from the group consisting of an insertion, a replacement and a deletion of at least one amino acid residue from the known amino-terminus."

Further, owing to an earlier restriction requirement in this case, the scope of claims 1-22 has been limited to ZYMV. Fernandez's teachings deal with plum pox virus (PPV). PPV appeared in claim 5, which has been withdrawn. The Examiner has, on the one hand attempted to exclude PPV from what is claimed while, on the other hand, relied upon a PPV research article to formulate an anticipation rejection. Applicant respectfully asserts that such a practice is not proper.

The Examiner's § 102(b) rejection based upon Fernandez is traversed.

§ 102(a) Rejections - Varrelmann

The Examiner has rejected claims 1,2,3,6-9,15 and 22 under §102(a) as being anticipated by Varrelmann et al. (Journal of Virology, 2000; hereinafter Varrelmann)

The objective of Varrelmann is to demonstrate the feasibility of mutating the core domain of a coat protein in a potyvirus (see Figure 1 of Varrelmann). Thus, Varrelmann, like

Fernandez, does not teach, hint or fairly suggest that insertion of a heterologous sequence at the amino-terminus, as instantly claimed, is necessary, advantageous, desirable or even feasible.

Again, the claims before the Examiner are limited to those vectors which include "*a heterologous nucleic acid sequence inserted at the amino-terminus of the potyvirus coat protein.*" [claim 1]. Applicant stresses that the claimed amino-terminus does not reside within the "core" of the CP as taught by Varrelmann..

Further, owing to an earlier restriction requirement in this case, the scope of claims 1-22 has been limited to ZYMV. Varrelmann's teachings deal with plum pox virus (PPV). PPV appeared in claim 5, which has been withdrawn. The Examiner has, on the one hand attempted to exclude PPV from what is claimed while, on the other hand, relied upon a PPV research article to formulate an anticipation rejection. Applicant respectfully asserts that such a practice is not proper.

The Examiner's §102(a) rejection based upon Varrelmann is traversed.

All §102 rejections are traversed.

§ 103(a) Rejections – Fernandez and others

The Examiner has rejected claims 1,3,4,6-15 and 19-22 under §103(a) as being obvious with respect to Fernandez in view of US 5,955,647 (hereinafter Fitchen) and further in view of Atreya et al. (PNAS, 1993; hereinafter Atreya).

The Applicant reiterates, owing to an earlier restriction requirement in this case, the scope of claims 1-22 has been limited to ZYMV. Fernandez's teachings deal with plum pox virus (PPV). PPV appeared in claim 5, which has been withdrawn.

Similarly, Fitchen teaches mutation of TMV. TMV is not a potyvirus. As such, any inference that what is true for TMV will be true for ZYMV is not valid. Further, Fitchen teaches modification of the amino-terminal domain, not the amino-terminus, as set forth hereinabove in relation to Fernandez and to Varrelmann.

Further, Atreya fails to teach "*a heterologous nucleic acid sequence inserted at the amino-terminus of the potyvirus coat protein.*" as instantly claimed.

Further, the Examiner has attempted to limit the claims to ZYMV while relying on non-ZYMV citations to formulate an obviousness rejection. Applicant respectfully asserts that such a practice is not proper, especially as regards the Fitchen reference which deals with a virus outside the potyvirus family.

In summary, none of the references hint or fairly suggest, whether alone or in combination, what is claimed.

The Examiner's rejection under §103(a) is traversed.

All rejections are traversed.

MPEP § 821.0-Right to Rejoinder

Applicant respectfully asserts that independent claims 23, 26 and 28, currently withdrawn, include all of the limits of claim 1. Because claim 1 is in condition for allowance, rejoinder of these withdrawn claims, and all claims which depend therefrom, is respectfully requested.

In view of the above amendments and remarks it is respectfully submitted that independent claims 1, and hence dependent claims 2-15 and 19-22 are in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited. Further, rejoinder of claims 23-35, and their allowance, is respectfully requested.

Respectfully submitted,

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APPENDIX A

THE POTYVIRIDAE

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sequences are summarized in Table 5.1 along with the corresponding reference citations and information on the coat protein size and the length of the 3′ non-coding region. In addition, comparative coat protein sequence analyses of 98 strains of 25 viruses have been made by high performance liquid chromatographic (HPLC) profiling of tryptic peptides combined with amino acid composition and sequence analysis of selected peptides (Table 5.2). This approach enables the sequence identity between groups of coat proteins to be estimated rapidly without resorting to the rigours of full protein sequence determination.

One feature that has frustrated amino acid sequence determinations of potyvirus coat proteins has been the presence of an N-terminal blocking group on some coat proteins but not others. Blocked N-terminal residues have been found for JGMV (Shukla *et al.*, 1987), TVMV (Domier *et al.*, 1986), three strains of PWV (Shukla *et al.*, 1988d), the HAT strain of TEV (Allison *et al.*, 1985a, 1986), SCMV-MDB (Frenkel *et al.*, 1991) and WSMV (Niblett *et al.*, 1991). In contrast, the NAT strain of TEV (Allison *et al.*, 1985a,b), the five strains of PVY (Shukla *et al.*, 1986, 1988c) including the pepper mottle strain (Dougherty *et al.*, 1985a), the D strain of PPV (Ravelonandro *et al.*, 1988) and the SC strain of SCMV (Frenkel *et al.*, 1991) had free N-termini and gave good data on automated sequencing. Comparison of the amino acid sequences (Figs 5.1–5.8) show that all blocked coat proteins start with S, while the unblocked proteins have A or G at their N termini. The nature of the blocked N-terminus is assumed to be acetyl-S as found for JGMV (Shukla *et al.*, 1987).

The coat proteins from distinct potyviruses vary considerably in size ranging from 251 amino acids for BaMMV to 332 amino acids for PPV-El Amar (Table 5.1). As shown in Figs 5.1–5.8 these size differences are largely due to variations at the N-terminal end of the coat protein. When the sequences are aligned for maximum identity these N-terminal regions range from 19 residues in BaMMV to 97 residues in PPV-El Amar. In contrast the C-terminal ends of the coat proteins vary in length by only one or two residues (Fig. 5.1). Exceptions are SAPV, where the last 10 residues include a four residue repeat (MTHG) making it longer, and BaYMV and BaMMV where the C-terminal region is seven residues shorter than the average.

There has been some doubt about the true N-terminus of the coat protein of the rymovirus WSMV. Attempts to sequence it by protein chemical means have been unsuccessful presumably because the N-terminal S residue is N-acetylated as for JGMV-JG (Shukla *et al.*, 1987). The cDNA sequence reveals five potential QS sites between NIb and the coat protein which would lead to the generation of coat proteins of 418, 322, 319, 307 and 288 amino acid residues with predicted molecular weights of 46.8 kDa, 35.7 kDa, 36 kDa, 34.3 kDa, 31.7 kDa respectively (Niblett *et al.*, 1991). This range of molecular weights is in good agreement with the patterns of 42–47 kDa, 36 kDa, 33 kDa, 32 kDa and 31 kDa bands seen on SDS-polyacrylamide gel electrophoresis (Brakke *et al.*, 1990; Niblett *et al.*, 1991). The smaller bands are considered to be further proteinase

breakdown products as they increase in proportion with time and all r with WSMV antibodies on Western blotting (Brakke *et al.*, 1990; Nil *et al.*, 1991). Examination of the partial cDNA sequence for WS (Niblett *et al.*, 1991) and its comparison with the aligned sequences of genomes of members of the *Potyvirus* and *Bymovirus* genera (Fig. 4.2, p. 80) reveals that the first putative QS cleavage site (418 residues i the coat protein C-terminus) falls within a highly conserved sequenc the NIb protein. This site is 79 residues downstream from the active GDD sequence, whereas the second QS site (yielding a 322 residue duct) is very close to the putative NIb–CP junctions found in all o potyviral polyproteins. Since the extent of C-terminal processing of NIb protein is not known, the occurrence of higher molecular we forms of WSMV coat protein suggest that upstream cleavages close the GDD active site sequence are tolerated. Electron micrographs re that WSMV particles are thicker (15 nm diameter) than most potyvir (Hollings and Brunt, 1981a) as might be expected if the coat protein sists of 418 residues and has a large N-terminal domain of 172 resi folded on the surface of the virus particle. A second example of mul NIb–CP cleavage sites is found with PRSV where two sites occu residues apart to yield two forms of coat protein (Yeh *et al.*, 1982).

The data in Fig. 5.1 show that the N-terminal ends of potyvirus proteins as a whole vary considerably in sequence. Key features in t sequences have been aligned as follows: the N-terminal S, A or G resi the DAG aphid-transmission triplet within 5–12 residues from thi terminus, and the KKDK type sequences that occur 1–7 residues d stream. The alignments in Fig. 5.1 also reveal small regions of sequ identity in the N-terminal region that are restricted to selected pai subgroups of sequences such as: (i) the alternating repeats of K a residues found in PRSV, PSbMV, TuMV, and MDMV-A; (ii) the P sequences in JGMV, OrMV, SPFMV, PPV and SCMV-MDB; (iii) the A K rich repeating sequences in the SrMV-SCH/BaYMV pair; and (iv GSGSG sequences in LMV-O, PWV-K, ZYMV-C, WMV2, and particu the SCMV-MDB/WSMV pair. As shown in the first block of sequenc Fig. 5.1 the N-terminal regions of the coat proteins of SCMV-MDB WSMV have quite strong sequence identity with 32 of the first 65 resi of WSMV having identical counterparts in SCMV-MDB. Similarly 26 o N-terminal 59 residues of BaYMV have identical counterparts in Sr SCH. Hammond (1992) has also examined these N-terminal sequences suggested that there are three major motifs: the long forms that have rich sequences as found in PPV and SCMV; the medium length forms are enriched in K/E, K/D or related residues as found in PVY, BY C1YVV, PWV, ZYMV, SbMV, PRSV and TuMV; and the short form f in OrMV which contains no K residues but is rich in P and G resi

In contrast to these variable N-terminal sequences, striking ide across all sequences commences around the trypsin cleavage site (Sh *et al.*, 1988b) beginning at the position equivalent to residue 30 i sequence KDKIDVNAG in PVY-D. This sequence identity extends thr

Table 5.2. Summary of coat protein HPLC profiles

Virus	Acronym	Reference
Tobacco etch virus	TEV-HAT	McKern *et al.*, 1990 McKern *et al.*, 1992a
Potato virus Y	PVY-D, -10, -18, -43*	Shukla *et al.*, 1986, 1988a,c McKern *et al.*, 1992a
Bean yellow mosaic	BYMV-GDD, G, G-81-1, F, K, RL7, S, BYMV-Scott	McKern *et al.*, 1993a MCKern *et al.*, 1992a, 1993a
Pea mosaic	PMV-204-1, -I, -Provvidenti	McKern *et al.*, 1993a
White lupin mosaic	WLMV	McKern *et al.*, 1993a
Clover yellow vein	ClYVV-B, C81, LI, Pratt, Washington	McKern *et al.*, 1992a, 1993a
Sweet pea mosaic	SPMV	McKern *et al.*, 1993a
Passionfruit woodiness	PWV-TB, -M, -S*	Shukla *et al.*, 1988a,d
Watermelon mosaic 2	WMV2	Shukla *et al.*, 1988a Jain *et al.*, 1992 McKern *et al.*, 1993b
Moroccan watermelon mosaic	WMV-Morocco†	McKern *et al.*, 1993b
Soyabean mosaic	SbMV-N	Jain *et al.*, 1992 McKern *et al.*, 1993b
	SbMV-G1, G2, G3, G4, G5, G6, G7, VA, SbMV-Brazil, O, 12/18, 75/16/1, Wis	Jain *et al.*, 1992 Jain *et al.*, 1992 McKern *et al.*, 1992a
Bean necrosis mosaic	BNMV-NL3, NL5, NL8, TN1	McKern *et al.*, 1992c
Bean common mosaic	BCMV-CH2, NL1, NL2, NL4, NL6, NL7, BCMV-PR1, RU1, US1, US2(D,P&Z), US3 BCMV-US4, US5, US6, US7, US10	McKern *et al.*, 1992c McKern *et al.*, 1992c McKern *et al.*, 1992c
Peanut stripe	PStV-Str, Blotch, Mild motle, China, PStV-T1, T2, T3, T6, T8,	McKern *et al.*, 1992a,b,c Kittipakorn *et al.*, 1993
Soyabean virus	PM, PN, 74	McKern *et al.*, 1992a
Azuki bean mosaic	AzMV	McKern *et al.*, 1992a
Blackeye cowpea mosaic	B1CMV-Type, Wisconsin	McKern *et al.*, 1992a,b,c
Peanut mottle	PeMoV-Poty-rape, Barnett	Kittipakorn *et al.*, 1993
Cowpea aphid-borne mosaic	CABMV-Morocco	McKern *et al.*, 1994
Papaya ringspot	PRSV-W	Jain *et al.*, 1992 McKern *et al.*, 1993b
Turnip mosaic	TuMV-	Haq *et al.*, 1994
Maize dwarf mosaic	MDMV A-Type, Texas,	McKern *et al.*, 1991b
Johnsongrass mosaic	JGMV-JG, KS1, O	Shukla *et al.*, 1987, 1988a McKern *et al.*, 1990, 1991b
Sugarcane mosaic	SCMV-A, O, Isis, SC, BC, Sabi, MDB	McKern *et al.*, 1990, 1991b Shukla *et al.*, 1987, 1988a
Sorghum mosaic	SrMV-SCH, SCM	McKern *et al.*, 1991b

* Includes *S. aureus* V8 peptide profiles; all others are tryptic peptide profiles.
† Formerly the Morocco strain of WMV2.

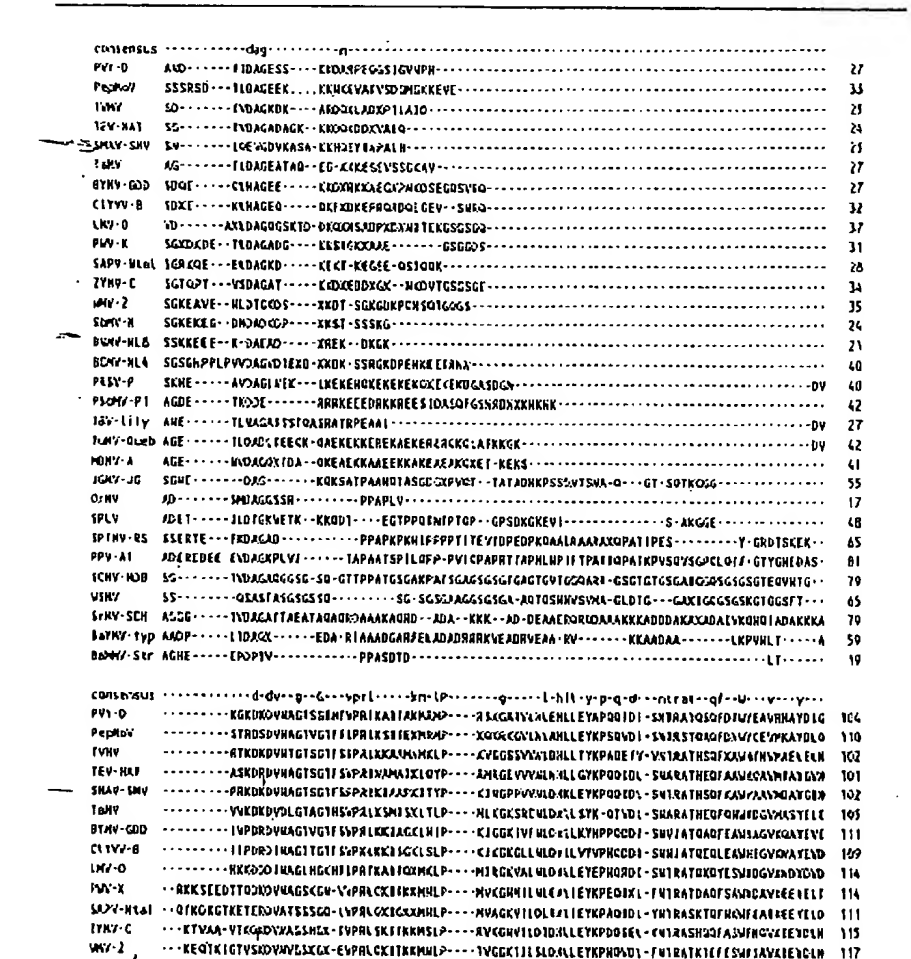
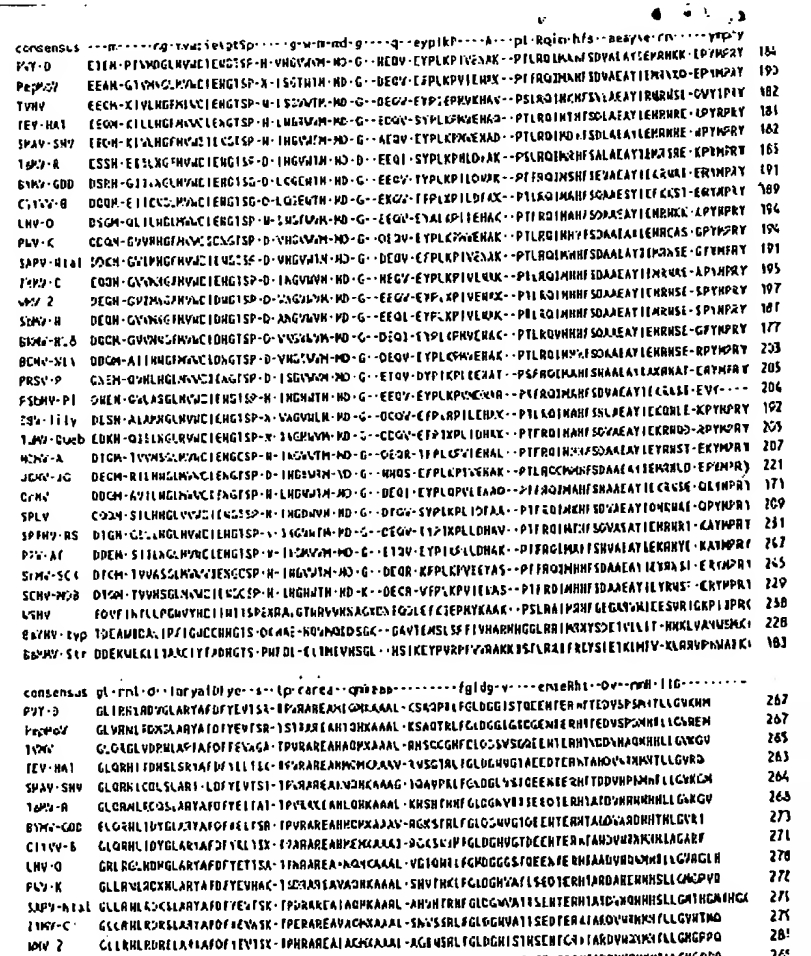


Fig. 5.1. Multiple alignment of the amino acid sequences of the coat proteins from 31 distinct potyviruses. The first 28 are from members of the *Potyvirus* genus; WSMV is a species of the *Rymovirus* genus and BaYMV and BaMMV are species of the *Bymovirus* genus. The data sources are listed in Table 5.1. A consensus sequence is given at the top of the figure where: uppercase letters represent invariant residues; and lower case letters represent nearly invariant residues. In the first block of sequences the listed sequence order has been changed to highlight the similarity between the



SCMV-MOB/WSMV and SrMV-SCH/BaYMV pairs. Additional information on comparative sequences l been obtained by HPLC peptide mapping as listed in Table 5.2. In this figure the N-terminus of th WSMV coat protein corresponds to the second of the five QS cleavage sites used. An additional : occurs 96 residues upstream and is shown in Chapter 4 (Fig. 4.2). PRSV is also reported to use second site 20 residues upstream from that shown here. The additional sequence is also shown Chapter 4 (Fig. 4.2).

to the C-terminus of the coat protein. A consensus sequence motif highlighting the conserved regions is shown along the top of each block of data in Fig. 5.1. There are 56 amino acid residues totally conserved among the coat protein sequences of the 26 aphid transmitted potyviruses with 12 of these also conserved in those of the mite- and fungus-transmitted potyviruses. As shown in the consensus sequence in Fig. 5.1, P residues feature frequently among the conserved residues and there is greater conservation in the C-terminal half of the coat protein than in the N-terminal half. Most sequences contain the C residue equivalent to 119 in PVY-D and some contain additional Cs. The conserved AFDF sequence (equivalent to 199–202 in PVY-D) is also found in the coat proteins of three other filamentous plant viruses, the potexviruses, carlaviruses and closteroviruses (Dolja *et al.*, 1991).

Coat proteins of virus strains

Primary structure data are also available for multiple strains of 17 of the aphid-transmitted potyviruses and two fungus-transmitted potyviruses (Table 5.1) and these will be discussed in detail in this section. The coat protein sequences for 22 strains of PVY from different host plants and different parts of the world are shown in Fig. 5.2. These CPs contain 267 amino acid residues except for that of PVY-18 which is one residue shorter having a deletion at position 25 (Shukla *et al.*, 1988c). The sequence for PVY-NZL is incomplete as no information is available regarding the first three amino acids (Hay *et al.*, 1989). All PVY strains contained only a single C residue (at position 119) and, where examined, did not have a blocked N terminus. The amino acid sequence of a pepper mottle virus isolate of unreported origin has been determined (Dougherty *et al.*, 1985a). As shown in Fig. 5.2 it has very high sequence identity with the other strains of PVY and on the basis of this homology it was suggested that PepMoV, originally described as an atypical strain of PVY (Zitter, 1972), should be considered a strain of PVY (Shukla *et al.*, 1986). Recently Vance *et al.* (1992a,b) have sequenced the coat protein (Fig. 5.1) and complete genome of an authentic isolate of PepMoV. These data show that PepMoV is a distinct potyvirus from PVY and from the pepper mottle strain of PVY sequenced by Dougherty *et al.* (1985a). HPLC profiles for coat protein peptides from four strains of PVY have also been established as shown in Table 5.2.

Van der Vlugt (1993) has analysed these 22 sequences and shown that they fall into two subgroups. Most of the viruses in the first subgroup (PVY-N11, N12, Jp, T, GO16, NZL, Hu and Russ) have been described as typical PVY^{n} ('necrosis') isolates (Van der Vlugt, 1993). The rest of the strains, with the exception of PVY-PepMo, were classified as typical PVY^{o} ('common') isolates and are more diverse. They can be further grouped into four clusters: the four Australian isolates (D, 10, 18 and 43); the six isolates (Fr, US, 02, 03, 04 and Ch); strain I on its own; and the

The sequences for four strains of BYMV are shown in Fig. 5.3, and reveal 15–33 differences between them. All are the same size (273 amino acids) and many of the substitutions are shared by other strains. The sequences for the coat proteins of three strains of ClYVV are also shown in Fig. 5.3. The ClYVV-30 strain is two residues longer than the others having a double insertion, VG, at positions 29 and 30. The New Zealand isolate has an additional C residue at position 181. The ClYVV strains differ from each other at 21–22 positions. HPLC profiles for several strains of BYMV and ClYVV have been compared with those of pea mosaic and white lupin mosaic viruses as summarized in Table 5.2 and revealed that the latter viruses are strains of BYMV, not distinct viruses (McKern *et al.*, 1993a). The complete sequence of PMV-I has confirmed this conclusion (Xiao *et al.*, 1994). As shown in Fig. 5.3 the PMV-I coat protein shows very high sequence identity (97%) to that of BYMV-CS with only eight differences between these two strains.

The sequences for strains of two other viruses that infect legumes, BNMV and BCMV, are shown in Fig. 5.4. The three strains of BNMV (formerly the serogroup A strains of BCMV) are very similar to each other with only five differences between BNMV-NL3 and NL5 and 7–10 differences between these two strains and NL8. BNMV-NL8 has a G to A mutation at the third position of the DAG triplet at residue 11 which would be expected to abolish aphid-transmission as found in the NAT strain and mutants of TVMV (Atreya *et al.*, 1990, 1991). The HPLC profile show that BNMV-TN1 is very similar to these three strains (McKern *et al.*, 1992c).

The coat protein sequences of BCMV-NL4 (Vetten *et al.*, 1992b), NL1 and NY15 (Khan *et al.*, 1993) are shown in Fig. 5.4 along with the coat protein sequences of BICMV-W (Khan *et al.*, 1993), PStV-Stripe (McKern *et al.*, 1991a) and PStV-Blotch (Cassidy *et al.*, 1993). The two PStV sequences differ at only two positions and are as similar to the BCMV and BICMV sequences as are the sequences of the accepted BCMV strains NL1, NY15 and NL4. Thus BCMV, BICMV and PStV are considered to be strains of the one potyvirus, BCMV (McKern *et al.*, 1992b,c). HPLC profiles for another 16 strains of BCMV and eight strains of PStV have confirmed this close relationship (Table 5.2). BCMV-NL1 and NY15 have an additional C residue at position 26 in the N-terminal region. The additional residue at position 216 in BCMV-NL4 is not shared by NL1, NY15, BICMV-W, PStV-Stripe or PStV-Blotch. BCMV-NL1 and NY15 have a G to S mutation at the third position of the DAG triplet. The mutation D to N in the first position of the DAG triplet of PStV-Stripe should have no effect on aphid transmission since this mutation was without effect on TVMV six specific mutants (Atreya *et al.*, 1991).

Coat protein sequences for three strains of SbMV, three strains of WMV2 and three strains of ZYMV are shown in Fig. 5.5 and reveal very few differences between the strains of each virus. Many of the differences involve residues in the DAG aphid transmission signal in the N-termin